

RELATIONSHIP OF TRIBEČ VIRUS TO TICK CELLS AND TISSUES

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Summary. — Tribeč virus persisted in parenterally inoculated half-engorged female *Ixodes ricinus* and *Dermacentor marginatus* ticks for at least 22 and 43 days, respectively (the longest intervals tested). The virus multiplied well also in *Hyalomma dromedarii* tick tissue cultures, reaching titres of up to $10^{4.5}$ mouse intracerebral (ic) LD₅₀/0.01 ml from 4—14 days after infection. The fluorescent antibody technique revealed the viral antigen in the form of confluent and brightly fluorescing granules in the cytoplasm of infected cells starting from the 2nd day.

Introduction

Tribeč virus was first isolated from ticks (Grešíková *et al.*, 1965). Although serologically classified as a member of the Kemerovo group of arboviruses, its transmission by an arthropod vector has not yet been verified. No host was yet found to develop viraemia high enough for infection of ticks. We attempted, therefore, to obtain information about the relationship of Tribeč virus to tick tissues in an artificial way. We studied virus multiplication in intraperitoneally infected half-engorged female ticks and in tick tissue cultures.

Materials and Methods

Virus. A lyophilized strain of Tribeč virus kept in the WHO Reference Laboratory for Arboviruses in Bratislava (Rajčáni and Grešíková, 1967) was used.

Ticks and tick tissue cultures. Persistence of virus was investigated in female *Ixodes ricinus* and *Dermacentor marginatus* ticks caught in areas known to be free of tick-borne encephalitis virus. The females, half-engorged on uninfected guinea pigs, were inoculated parenterally (Řeháček, 1966) and then kept at room temperature in Erlenmeyer flasks.

Tissue cultures from *Hyalomma dromedarii* ticks were prepared as previously described (Řeháček, 1965a). The cells were seeded on coverslips in tubes, and the monolayers formed in 3—5 days were infected with 0.1 ml of virus without washing. The inoculum, prepared from the stock material, contained 10^7 mouse LD₅₀ of Tribeč virus. Three infected ticks were taken at intervals of 2—4 days and 10% suspensions were prepared from them in Earle's solution with 5% foetal calf serum and antibiotics. Tenfold dilutions of the suspensions were ic inoculated into suckling mice, using 8 animals for each dilution. From the infected tissue cultures, 0.1 ml samples of the medium were taken at the same intervals and the virus titres were determined in mice as with ticks.

The fluorescent antibody technique was used only with tick tissue cultures. At least 2 coverslips were taken at the same intervals as the samples of medium (see above), fixed in acetone and stained by the indirect method with immune mouse serum against Tribeč virus and anti-mouse conjugate. The procedure was described in detail previously (Rajčáni and Grešíková, 1967).

Uninfected coverslips, treated similarly to the infected ones, were used as controls at each interval. To eliminate nonspecific fluorescence, the serum and the conjugate were absorbed with a 20% suspension of *Hyalomma* nymphs before staining.

Results

Persistence of Tribeč virus in ticks

Half-engorged female ticks, 50–100 of either species, were inoculated intraperitoneally with $10^{6.5}$ mouse LD_{50} of Tribeč virus in 0.01 ml volumes. In *D. marginatus* females the virus was still found on the 43th day after

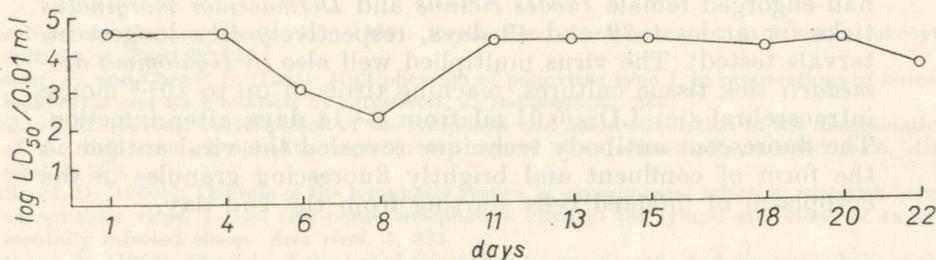


Fig. 1.

Persistence of Tribeč virus in *Ixodes ricinus* females

inoculation. From the 15th till the 43th day it was irregularly detectable in titres of up to 10^3 mouse LD_{50} per 0.01 ml of the tick suspension (the experiment lasted from May 15 to June 12). In *I. ricinus* females, the virus persisted for 22 days (from July 1 to 22) without deviations in the titre levels except of a decrease between the 4th–11th day after infection (Fig. 1).

Multiplication of Tribeč virus in tick tissue cultures

Four experiments were carried out, but only the second one, in which the quality of cell monolayers was the best, will be described.

The amount of virus in the medium dropped at 24 hours after inoculation to undetectable values. Starting from the 2nd post-infection day we found a titre increase of about 4 log units and these values were detectable until the 14th day, i.e. until the end of the experiment (Fig. 2).

In parallel with infectivity titrations, the occurrence of viral antigen in the infected cells was traced by the fluorescent antibody method. A clear-cut specific fluorescence was seen in the cytoplasm from the 2nd to 7th day after infection. At the beginning only some fluorescing granules with perinuclear localization were found (Fig. 3), but by the 4th day they increased in number and blended together into large, bright fluorescing masses, filling the whole cytoplasm (Fig. 4). The nuclei remained free of antigen all the time. The viral antigen was found in both epithelial-like and fibroblast-like cells. At the later intervals, between the 7th to 14th day, the cells showed various degenerative changes, especially vacuolation of the cytoplasm and

cytolysis. Some cells were completely disintegrated and formed structureless masses. These also showed occasionally a positive fluorescence (Fig. 5). No fluorescence was observed in the uninfected cells, even in those showing degenerative changes at the late intervals of cultivation.

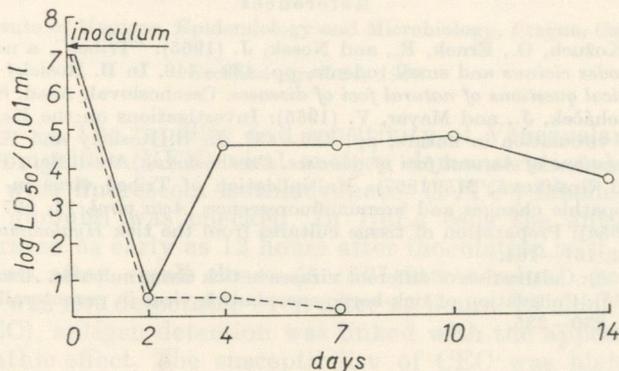


Fig. 2.

Cultivation of Tribeč virus in tick tissue cultures

Discussion

The rapid decrease in the virus titre in the medium soon after inoculation was also observed in control tubes, which contained no cells. This decrease was due to the thermal inactivation of the thermolabile Tribeč virus. Some virions, however, before being inactivated, were adsorbed onto the cells and started to multiply. This was proved by the occurrence of the viral antigen in the cytoplasm of the infected cells and by the presence of infective virus in the medium.

The persistence of Tribeč virus in ticks was similar to that of Kemerovo virus in the same arthropods (Libíková *et al.*, 1965), pointing out the relationship of the two viruses. The results obtained with tick-borne encephalitis virus under the same conditions were quite different (Řeháček, 1966).

The detection by the fluorescent antibody technique of the viral antigen in the tick cell cultures was attempted in parallel with the titration of extracellular virus. According to the intensity of the cytoplasmic fluorescence it can be assumed that also the amount of intracellular infective virus surpassed the critical value of 10^4 mouse LD₅₀ per 0.01 ml cell suspension, necessary for the immunofluorescent detection of Tribeč virus in chick embryo cells (Rajčáni and Grešíková, 1967).

When comparing the results obtained with the infection of tick tissue cultures with several viruses, according to which practically only the arboviruses were able to multiply in them (Řeháček, 1965*b*), the reproduction of Tribeč virus in these cells supports its inclusion into the arbovirus group. Nevertheless, we consider this marker only as a preliminary one and disputable.

The present results showed that tick tissues and cells provide a favourable environment for Tribeč virus reproduction. This, however, is a prerequisite, but in no case a proof for considering ticks as natural vectors or reservoirs of Tribeč virus.

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Explanation of Photomicrographs:

Specific fluorescence of Tribeč virus antigen in tick tissue cells. $\times 400$.

Fig. 3. Granular cytoplasmic fluorescence in a fibroblast-like cell, 4th day after inoculation.

Fig. 4. Confluent cytoplasmic fluorescence in the cytoplasm of an epithelial-like cell; the nucleus remains free of antigen; 7th day after inoculation.

Fig. 5. A large fibroblast-like cell in the center and a badly outlined degenerated cell at the periphery, both with bright granular cytoplasmic fluorescence. Also some cell debris shows positive fluorescence; 7th day after inoculation.